THE EVOLUTION OF THE CELL. II

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Even more remarkable than the relation of the chromosomes to cell-reproduction is their behavior in relation to sexual phenomena. In the life-cycles of Metazoa the sexual act consists of the fusion of male and female pronuclei, each containing a definite and specific number of chromosomes, the same number usually, though not always, in each pronucleus. It has been established in many cases, and it is perhaps universally true, that in the act of fertilization the male and female chromosomes remain perfectly distinct and separate in the synkaryon or nucleus formed by the union of the two pronuclei, and, moreover, that they continue to maintain and to propagate their distinct individuality in every subsequent cell-generation of the multicellular organism produced as a result of the sexual act. In this way, every cell of the body contains in its nucleus distinct chromatinic elements which are derived from both male and female parents and which maintain unimpaired their distinct and specific individuality through the entire life-cycle. This distinctness is apparent at least in the germ-cell-cycle of the organism, but may be obscured by secondary changes in the nuclei of the specialized tissue-cells.

Only in the very last stage of the life-cycle do the group of male and female chromosomes modify their behavior in a most striking manner. In the final generation of oogonia or spermatogonia, from which arise the oocytes and spermatocytes which in their turn produce the gamete-cells, it is observed that the male and female chromosomes make a last appearance in their full number, and then fuse in pairs, so as to reduce the number of chromosomes to half that previously present.

In *Aggregata* also Dobell and Jameson have shown that the union of the pronuclei in fertilization brings together two sets each of six chromosomes, and that these then fuse with one another in pairs according to type, that is to say *a* with *a*, *b* with *b*, *c* with *c*, and so on. Analogous phenomena have been demonstrated also in the gregarine *Diplospora*. We have here a difference in detail, as compared with the Metazoa, in that the fusion takes place at the fertilization and not as the first step in the maturation of the germ-cells; but in both cases alike the fusion of chromatin-elements individually distinct and exhibiting specific characteristics is to be regarded as the final consummation of the sexual act, though long deferred in the Metazoan life-cycle.

As Vejdovský has pointed out, there can be no more striking evidence of the specific individuality of the chromosomes than their fusion or copulation in relation to the sexual act. Is there any other constant element or constituent of living organisms exhibiting to anything like the same degree the essentially vital characteristics of individuality manifested in specific behavior? If there is, it remains to be discovered.

I come now to the question of the permanence and immortality, in the biological sense of the word, of the chromatinic particles, which may be summarily stated as follows: the chromatinic particles are the only constituents of the cell which maintain persistently and uninterruptedly their existence throughout the whole life-cycle of living organisms universally.

I hope I shall not be misunderstood when I enunciate this apparently sweeping and breathless generalization. I am perfectly aware that in the life-cycle of any given species of organism there may be many cell-constituents besides the chromatin-particles that are propagated continuously through the whole life-cycle; but cell-elements which appear as constant parts of the organization of the cell throughout the life-cycle in one type of organism may be wanting altogether in other types. With the exception

of the chromatin-particles there is no cell-constituent that can be claimed to persist throughout the life-cycles of organisms universally. To take some concrete examples; the cytoplasmic grains known as mitochondria or chrondriosomes have been asserted to be persistent elements throughout the germ-cycle of Metazoa, and the function of being the bearers of hereditary tendencies has been ascribed to them. But Vejdovský[22] flatly denies the alleged continuity in cases investigated by him, and though chrondriosomes have been described in some Protozoa, there is no evidence whatever that they are of universal occurrence in Protista. Centrosomes, intranuclear or extranuclear, have been stated to be constant cell-components in some organisms; whether that is true or not it seems quite certain that in many organisms the cells are entirely without centrosomic bodies of any kind, as for example in the whole group of Phanerogams. So it is with any other cell-constituent that can be named. It may be that this is only the result of our incomplete knowledge at the present time. I am prepared, however, to challenge anyone to name or to discover any cell-constituent, other than the chromatinic particles, which are present throughout the life-cycle, not merely of some particular organism, but of organisms universally.

In this feature of continuity the chromatin-constituents of the cell present a remarkable analogy with the germ-plasm of Metazoa. Just as the germ-cells of Metazoa go on in an uninterrupted, potentially everlasting series of cell-generations, throwing off, as it were, at each sexual crisis a soma which is doomed to but a limited lease of life, during which it furnishes a nutritive environment for further generations of germ-cells; so in the series of cell-generations themselves, whether in the germ-cell-cycles of Metazoa or in the life-cycles of Protista the chromatin-particles maintain an uninterrupted propagative series within a cell-body of which the various parts have a limited duration of existence, making their appearance, flourish-

[22] *L. c.*, pp. 77–89.

ing for a time, and disappearing again. This analogy between the chromatin of cells and the germ-plasm of multicellular organisms becomes still more marked when we find that in many Protozoa the chromatin may undergo a specialization into generative and trophic chromatin, the former destined to persist from one life-cycle to another, the latter destined to control cell-activities merely during one cycle, without persisting into the next. The differentiation of generative and trophic chromatin is now well known to occur in many Protozoa, and in its most extreme form, as seen in the Infusoria, it is expressed in occurrence of two distinct nuclei in the cell-body.

To recapitulate my argument in the briefest form; the chromatinic constituents of the cell contrast with all the other constituents in at least three points: physiological predominance, especially in constructive metabolism; specific individualization; and permanence in the sense of potential biological immortality. Any of these three points, taken by itself, is sufficient to confer a peculiar distinction to say the least, on the chromatin-bodies; but taken in combination they appear to me to furnish overwhelming evidence for regarding the chromatin-elements as the primary and essential constituents of living organisms, and as representing that part of a living body of any kind which can be followed by the imagination, in the reverse direction of the propagative series, back to the very starting-point of the evolution of living beings.

In the attempt to form an idea as to what the earliest type of living being was like, in the first place, and as to how the earliest steps in its evolution and differentiation came about, in the second place, we have to exercise the constructive faculty of the imagination guided by such few data as we possess. It is not to be expected, therefore, that agreement can be hoped for in such speculations; it would indeed be very undesirable, in the interests of science, that there should be no conflict of opinion in theories which, by their very nature, are beyond any possibility of direct verification at the present time. The

views put forward by any man do but represent the visions conjured up by his imagination, based upon the slender foundation of his personal knowledge, more or less limited, or intuition, more or less fallacious, of an infinite world of natural phenomena. Consequently such views may be expected to diverge as widely as do temperaments. If, therefore, I venture upon such speculations, I do so with a sense of personal responsibility and as one wishing to stimulate discussion rather than to lay down dogma.

To me, therefore, the train of argument that I have set forth with regard to the nature of the chromatinic constituents of living organisms appears to lead to the conclusion that the earliest living beings were minute, possibly ultramicroscopic particles which were of the nature of chromatin. How far the application of the term chromatin to the hypothetical primordial form of life is justified from the point of view of substance, that is to say in a biochemical sense, must be left uncertain. In using the term chromatin I must be understood to do so in a strictly biological sense, meaning thereby that these earliest living things were biological units or individuals which were the ancestors, in a continuous propagative series, of the chromatinic grains and particles known to us at the present day as universally-occurring constituents of living organisms. Such a conception postulates no fixity of chemical nature; on the contrary, it implies that as substance the primitive chromatin was highly inconstant, infinitely variable, and capable of specific differentiation in many divergent directions.

For these hypothetical primitive organisms we may use Mereschkowsky's term biococci. They must have been free-living organisms capable of building up their living bodies by synthesis of simple chemical compounds. We have as yet no evidence of the existence of biococci at the present time as free-living organisms; the nearest approach to any such type of living being seems to be furnished by the organisms known collectively as Chlamydozoa, which up to the present have been found to occur

only as pathogenic parasites. In view, however, of the minuteness and invisibility of these organisms, it is clear that they could attract attention only by the effects they produce in their environment. Consequently the human mind is most likely to become aware in the first instance of those forms which are the cause of disturbance in the human body. If free-living forms of biococci exist, as is very possible and even probable, it is evident that very delicate and accurate methods of investigation would be required to detect their presence.

I am well aware that the nature and even the existence of the so-called Chlamydozoa is uncertain at the present time, and I desire to exercise great caution in basing any arguments upon them. In the descriptions given of them, however, there are some points which, if correctly stated, seem to me of great importance. They are alleged to appear as minute dots, on the borderline of microscopic visibility or beyond it; they are capable of growth, so that a given species may be larger or smaller at different times; their bodies stain with the ordinary chromatin-stains; and they are stated to reproduce themselves by a process of binary fission in which the body becomes dumbbell-shaped, appearing as two dots connected by a slender thread, which is drawn out until it snaps across and then the broken halves of the thread are retracted into the daughter-bodies. This mode of division, strongly reminiscent of that seen in centrioles, appears to me to permit of certain important conclusions with regard to the nature of these bodies; namely, that the minute dot of substance has no firm limiting membrane on the surface and that it is of a viscid or semi-fluid consistence.

If it be permissible to draw conclusions with regard to the nature of the hypothetical biococci from the somewhat dubious, but concrete data funished by the Chlamydozoa, the following tentative statements may be postulated concerning them. They were (or are) minute organisms, each a speck or globule of a substance similar in its reactions to chromatin. Their substance could be described

as homogeneous with greater approach to accuracy than in the case of any other living organism, but it is clear that no living body that is carrying on constructive and destructive metabolism could remain for a moment perfectly homogeneous or constant in chemical composition. Their bodies were not limited by a rigid envelope or capsule. Reproduction was affected by binary fission, the body dividing into two with a dumbbell-shaped figure. Their mode of life was vegetative, that is to say, they reacted upon their environmental medium by means of ferments secreted by their own body-substance. The earliest forms must have possessed the power of building up their protein-molecules from the simplest inorganic compounds; but different types of biococci, characterized each by specific reactions and idiosyncrasies, must have become differentiated very rapidly in the process of evolution and adaptation to divergent conditions of life.

Consideration of the existing types and forms of living organisms shows that from the primitive biococcal type the evolution of living things must have diverged in at least two principal directions. Two new types of organisms arose, one of which continued to specialize further in the vegetative mode of life, in all its innumerable variations, characteristic of the biococci, while the other type developed an entirely new habit of life, namely a predatory existence. I will consider these two types separately.

(1) In the vegetative type the first step was that the body became surrounded by a rigid envelope. Thus came into existence the bacterial type of organism, the simplest form of which would be a Micrococcus, a minute globule of chromatin surrounded by a firm envelope. From this familiar type an infinity of forms arises by processes of divergent evolution and adaptation. With increase in size of the body the number of chromatin-grains within the envelope increase in number, and are then seen to be imbedded in a ground-substance which is similar to cytoplasm, apparently, and may contain non-chromatinic en-

closures. With still further increase of size the chromatin-grains also increase in number and may take on various types of arrangement in clumps, spherical masses, rodlets, filaments straight or twisted in various ways, or even irregular strands and networks,[23] and the cytoplasmic matrix, if it is correct to call it so, becomes correspondingly increased in quantity. I will not attempt, however, to follow up the evolution of the bacterial type further, nor to discuss what other types of living organisms may be affiliated with it, as I have no claims to an expert knowledge of these organisms. I prefer to leave to competent bacteriologists and botanists the problem of the relationships and phylogeny of the Cyanophyceæ, Spirochætes, etc., which have been regarded as having affinities with Bacteria.

(2) In the evolution from the biococcus of the predatory type of organism, the data at our disposal appear to me to indicate very clearly the nature of the changes that took place, as well as the final result of these changes, but leave us in the dark with regard to some of the actual details of the process. The chief event was the formation, round the biococci of an enveloping matrix of protoplasm for which the term periplasm (Lankester) is most suitable. The periplasm was an extension of the living substance which was distinct in its constitution and properties from the original chromatinic substance of the biococcus. The newly-formed matrix was probably from the first a semi-fluid substance of alveolar structure and possessed two important capabilities as the result of its physical structure; it could perform streaming movements of various kinds, more especially amœboid movement; and it was able to form vacuoles internally. The final result

[23] See especially Dobell, "Contributions to the Cytology of the Bacteria," *Quart. Journ. Micr. Science,* LVI (1911), pp. 461, 462. I can not follow Dobell in applying the term "nuclei" to these various arrangements of the chromatin-grains in Bacteria. Vejdovsky compares them with chromosomes; but there is no evidence that they play the part in the division and distribution of the chromatin-grains which is the special function of chromosomes, as will be discussed in more detail presently.

of these changes was a new type of organism which, compared with the original biococci, was of considerable size, and consisted of a droplet of alveolar, amœboid periplasm in which were imbedded a number of biococci. Whether this periplasm made its first appearance around single individual biococci, or whether it was from the first associated with the formation of zooglœa-like colonies of biococci, must be left an open question.

Thus arose in the beginning the brand of Cain, the prototype of the animal, that is to say, a class of organism, which was no longer able to build up its substance from inorganic materials in the former peaceful manner, but which nourished itself by capturing, devouring, and digesting other living organisms. The streaming movements of the periplasm enabled it to flow round and engulf other creatures; the vacuole-formation in the periplasm enabled it to digest and absorb the substance of its prey by the help of ferments secreted by the biococci. By means of these ferments the ingested organisms were killed and utilized as food, their substance being first broken down into simpler chemical constituents and then built up again into the protein-substances composing the body of the captor.

A stage of evolution is now reached which I propose to call the pseudo-moneral or cytodal stage, since the place of these organisms in the general evolution of life corresponds very nearly to Haeckel's conception of the Monera as a stage in the evolution of organisms, though not at all to his notions with regard to their composition and structure. The bodies of these organisms did not consist of a homogeneous albuminous "plasson," but of a periplasm corresponding to the cytoplasm of the cell, containing a number of biococci or chromatin-grains. Thus their composition corresponded more clearly to that of plasson as conceived by Van Beneden, when he wrote: 'Si un noyau vient à disparaître dans une cellule, si la cellule redevient un cytode, les éléments chimiques du noyau et du nucléole s'étant repandus dans le protoplasme, le plasson se trouve

de nouveau constituté.' If we delete from this sentence the word "chimiques" and also the words "et du nucléole," and substitute for the notion of the chemical solution of the chromatin-substance that of scattered chromatin-grains in the periplasm, we have the picture of the cytodal stage of evolution such as I have imagined it. It should be borne in mind that the ultimate granules of chromatin are probably in many cases ultra-microscopic; consequently they might appear to be dissolved in this cytoplasm when really existing as discrete particles.

In the life-cycles of Protozoa, especially of Rhizopods, it is not at all infrequent to find developmental phases which reproduce exactly the picture of the pseudo-moneral stage of evolution, phases in which the nucleus or nuclei have disappeared, having broken up into a number of chromatin-grains or chromidia scattered through the cytoplasm. We do not know as yet of any Protozoa, however, which remain permanently in the cytodal stage, that is to say, in which the chromatin-grains remain permanently in the scattered chromidial condition, without ever being concentrated and organized into true nuclei; but it is quite possible that some of the primitive organisms known as Proteomyxa will be found to exhibit this condition and to represent persistent Pseudo-monera or cytodes.

The next stage in evolution was the organization of the chromatin-grains (biococci) into a definite cell-nucleus. This is a process which can be observed actually taking place in many Protozoa in which "secondary" nuclei arise from chromidia. It seems not unreasonable to suppose that a detailed study of the manner in which secondary nuclei are formed in Protozoa will furnish us with a picture, or rather series of pictures, of the method in which the cell-nucleus arose in phylogeny. To judge from the data supplied by actual observation, the evolution of the nucleus, though uniform in principle, was sufficiently diversified in the details of the process. As one extreme we have the formation of a dense clump of small, separate

chromatin-grains, producing a granular nucleus of the type seen in Dinoflagellates, in Hæmogregarines, and in Diatoms. Amongst the chromatin-grains there may be present also one or more grains or masses of plastin forming true nucleoli. At the opposite extreme a clump of chromatin-grains becomes firmly welded together into a single mass in which the individual grains can no longer be distinguished, forming a so-called karyosome, consisting of a basis of plastin cementing or imbedding the chromatin-grains into a mass of homogeneous appearance. Whatever the type of nucleus formed, the concentration of the chromidia into nuclei does not necessarily involve all the chromidia, many of which may remain free in the cytoplasm.

In the chromidial condition the chromatin-grains scattered in the cytoplasm are lodged at the nodes of the alveolar framework.[24] Consequently a supporting framework of cytoplasmic origin, the foundation of the linin-framework, was probably a primary constituent of the cell-nucleus from the first. In many nuclei of the karyosomatic type it is very difficult to make out anything of the nature of a framework, which, however, in other cases is seen clearly as delicate strands radiating from the karyosome to the wall of the vacuole in which the karyosome is suspended. Probably such a framework is present in all cases, and each supporting strand is to be interpreted as the optical section of the partition between two protoplasmic alveoli.

With the formation of the nucleus the cytode or pseudomoneral stage has become a true cell of the simplest type, for which I propose the term *protocyte*. It is now the starting-point of an infinite series of further complications and elaborations in many directions. It is clearly

[24] Cf. Dobell, "Observations on the Life-History of Cienkowski's *Arachnula*," *Arch. Protistemkunde*, XXXI (1913), p. 322. The author finds that in *Arachnula* each nucleus arises from a single chromatin-grain, which grows to form a vesicular nucleus. Since the fully-formed nucleus contains numerous grains of chromatin, the original chromidiosome must multiply in this process.

impossible that I should do more than attempt to indicate in the most summary manner the various modifications of the cell-type of organism, since to deal with them conscientiously would require a treatise rather than an address, and, moreover, many such treatises exist already. The most conspicuous modifications of cell-structure are those affecting the periplasm, or, as we may now term it, the cytoplasm. In the first place, the cell as a whole takes various forms; primitively a little naked mass of protoplasm tending to assume a spherical form under the action of surface-tension when at rest, the cell-body may acquire the most diverse specific forms maintained either by the production of envelopes or various kinds of exoskeletal formations on the exterior of the protoplasmic body, or of supporting endoskeletal structures formed in the interior. The simple amœboid streaming movements become highly modified in various ways or replaced by special locomotor mechanisms or organs, flagella, cilia, etc., of various kinds. The internal alveolar cytoplasm develops fibrillæ and other structures of the most varied nature and function, contractile, skeletal, nervous, and so forth. The vacuole-system may be amplified and differentiated in various ways and the cytoplasm acquires manifold powers of internal or external secretion. And finally the cytoplasm contains enclosures of the most varied kind, some of them metaplastic products of the anabolic or catabolic activity essential to the maintenance of life, others of the nature of special cell-organs performing definite functions, such as centrosomes, plastids, chromatophores, etc., of various kinds.

With all the diverse modifications of the cytoplasmic cell-body the nucleus remains comparatively uniform. It may indeed vary infinitely in details of structure, but in principle it remains a concentration or aggregation of numerous grains of chromatin supported on some sort of framework over which the grains are scattered or clumped in various ways, supplemented usually by plastin or nucleolar substance either as a cementing ground-sub-

stance or as discrete grains, and the whole marked off sharply from the surrounding cytoplasm, with or without a definite limiting membrane. There is, however, one point in which the nucleus exhibits a progressive evolution of the most important kind. I refer to the gradual elaboration and perfection of the reproductive mechanism, the process whereby, when the cell reproduces itself by fission, the chromatin-elements are distributed between the two daughter-cells.

The chromatin-constituents of the cell are regarded, on the view maintained here, as a number of minute granules, each representing a primitive independent living individual or biococcus. To each such granule must be attributed the fundamental properties of living organisms in general; in the first place metabolism, expressed in continual molecular change, in assimilation and in growth, with consequent reproduction; in the second place specific individuality. As the result of the first of these properties the chromatin-granules, often perhaps ultra-microscopic, may be larger or smaller at different times, and they multiply by dividing each into two daughter-granules. As a result of the second property, chromatin-granules in one and the same cell may exhibit qualitative differences and may diverge widely from one another in their reactions and effects on the vital activities of the cell. The chromatin-granules may be either in the form of scattered chromidia or lodged in a definite nucleus. When in the former condition, I have proposed the term chromidiosome[25] for the ultimate chromatinic individual unit; on the other hand, the term chromiole is commonly in use for the minute chromatin-grains of the nucleus. The terms chromidiosome and chromiole distinguish merely between the situation in the cell, extranuclear or intranuclear, of the individual chromatin-grain or biococcus.

[25] "*Introduction of the Study of the Protozoa,*" Arnold, 1912, p. 65.